After inoculation, all eggs shall be incubated at 37°C±1°C for 48 hours.

(3) *Estimation of potency.* Only membranes from living embryos shall be removed and the number of specific lesions thereon shall be counted and recorded. The number of pock forming units in 1.0 ml. of vaccine shall be calculated from the number of lesions, the dilution factor and the volume used, to determine the titer of the undiluted vaccine. The accuracy of the titration shall be confirmed in each test by performing simultaneously the same type of titration with the reference vaccine which shall demonstrate its assigned titer.

(4) *Potency requirements*—(i) *Vaccine intended for multiple pressure administration.* Vaccine intended for multiple pressure administration shall have a titer at least equivalent to the reference vaccine.

(ii) *Vaccine intended for jet injection.* Vaccine intended for administration by jet injector shall have a number of pock forming units in one human dose at least equivalent to that contained in 0.1 ml. of the reference vaccine diluted 1:30.

(iii) *Heated liquid vaccine.* Samples of liquid vaccine from final containers taken at random shall be incubated at 35° to 37° C. for at least 18 hours, after which the heated sample shall be tested in parallel with a sample of unheated vaccine of the same lot, as prescribed in this paragraph. The vaccine is satisfactory if the heated sample retains at least one tenth of the potency of the unheated sample.

(iv) *Heated dried vaccine.* Samples of dried vaccine from final containers taken at random shall be incubated at 35° to 37° C. for 30 days, after which the heated sample shall be tested in parallel with a sample of unheated vaccine of the same lot, as prescribed in this paragraph. The vaccine is satisfactory if the heated sample retains at least one-tenth of the potency of the unheated sample.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 51010, Nov. 19, 1976]

§ 630.74 Tests for safety.

(a) *Anaerobes.* A 10-milliliter sample representative of the homogenized viral harvest or pool of several viral harvests shall be tested for the presence of anaerobes in the following manner: Before the addition of preservatives other than glycerin, the test sample shall be inoculated into freshly heated Fluid Thioglycollate Medium using a ratio of inoculum to culture medium sufficient for optimal bacterial growth. The test vessels shall be incubated at 35° to 37° C and observed daily for 10 days for evidence of bacterial growth. If bacterial growth is observed, the organism(s) shall be identified as to genus. Within 24 to 48 hours of an indication that there may be anaerobic growth, 1.0-milliliter samples from each vessel showing growth shall be inoculated subcutaneously into each of at least three mice weighing not more than 20 grams each, and into each of three guinea pigs weighing not more than 350 grams each. The animals shall be observed daily for 6 days for signs of tetanus or presence of other anaerobes. If the animals show no signs of tetanus or presence of other anaerobes, additional groups of the same types and numbers of animals shall be injected 9 days after evidence of anaerobic bacterial growth is observed in the original planting with 1.0-milliliter samples from each test vessel showing growth. The animals shall be observed daily for 6 days for signs of tetanus or presence of other anaerobes. If any animals die within 3 days without having shown signs of tetanus or presence of other anaerobes, the test shall be repeated within 18 hours of the deaths, with 0.1-milliliter samples of the culture from which that animal was inoculated. Samples from the culture shall be injected into each of three additional test animals of the same species, and the animals shall be observed daily for 6 days. If there is any evidence of the presence of pathogenic anaerobes, the viral harvest may not be used in the manufacture of Smallpox Vaccine.

(b) [Reserved]

(c) *Coliform organisms.* A 5.0 ml. sample of bulk vaccine shall be tested for the presence of coliform organisms by the method published by the American Public Health Association, Inc., in ''Standard Methods for the Examination of Water and Wastewater'' (13th edition, 1971), section entitled ''Multiple-Tube Fermentation Technic for

Members of the Coliform Group,'' pages 662–678 and any amendments or revisions thereof, which section is hereby incorporated by reference and deemed published herein. Said publication is available at most medical and public libraries and copies of the pertinent section will be provided to any manufacturer affected by the provisions of this part upon request to the Director, Center for Biologics Evaluation and Research, or to the appropriate Information Center Officer listed in 45 CFR part 5. In addition, an official historic file of the material incorporated by reference is maintained in the Office of the Director, Center for Biologics Evaluation and Research, or available for inspection at the Office of the Federal Register, 800 North Capitol Street NW., suite 700, Washington, DC 20408. A method different than that contained in the above cited section may be used to test for the presence of coliform organisms upon a showing that it is of equal or greater sensitivity. The ratio of the volume of inoculum to the volume of culture medium shall be such as will dilute the preservative to a level that does not inhibit growth of contaminating organisms. The vaccine is satisfactory if there is no evidence of coliform organisms.

(d) *Hemolytic streptococci and coagulase-positive staphylococci.* Each of three 1.0 ml. samples of bulk vaccine shall be spread uniformly on the surface of separate blood agar plates. The plates shall be incubated for 48 hours at 35° to 37° C. The vaccine is satisfactory if there is no evidence of the presence of either hemolytic streptococci or coagulase-positive staphylococci.

(e) *Viable bacteria*—(1) *Vaccine intended for multiple pressure administration.* Samples of each lot of both bulk and final container vaccine shall be tested for viable bacteria by a procedure designed to detect both aerobic and anaerobic growth through a period of 7 days. At least three 1.0 ml. samples of bulk vaccine and three 0.2 ml. samples of vaccine derived from not less than three final containers or dilutions thereof shall be inoculated into a volume of culture medium sufficient for optimal bacterial growth. The vaccine is satisfactory if it contains no more than 200 viable organisms per ml.

(2) *Vaccine intended for jet injection.* Samples of each lot of both bulk and final container vaccine shall be tested for viable bacteria in Fluid Thioglycollate Medium prepared in accordance with §610.12(e)(1)(i) of this chapter for at least a 7-day test period. A sample of at least 10.0 ml. of bulk vaccine and 1.0 ml. from each of at least 20 final containers shall be tested. The ratio of the volume of the inoculum to the volume of culture medium shall be such as will dilute the preservative in the inoculum to a level that does not inhibit growth of contaminating micro-organisms. The vaccine is satisfactory if it contains no more than one organism per 100 doses of vaccine.

(f) *Sterile vaccine.* The tests prescribed in paragraphs (c), (d), and (e) of this section need not be performed on a lot of Smallpox Vaccine that meets the sterility requirements prescribed in §610.12 of this chapter.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 51010, Nov. 19, 1976; 47 FR 9397, Mar. 5, 1982; 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§630.75 General requirements.

(a) *General safety.* Each lot of vaccine shall be tested for safety as prescribed in §610.11 of this chapter and shall meet the safety requirements of that section, except that for liquid Smallpox Vaccine distributed in capillaries, the test may be performed with a sample of bulk vaccine taken at the time of filling into final containers.

(b) *Preservative.* A preservative that meets the requirements of §610.15 of this chapter may be used, provided that if the preservative is phenol, its concentration shall not exceed 0.5 percent.

(c) *Labeling.* In addition to complying with all other applicable labeling provisions of this subchapter the package label shall bear the following:

(1) *Vaccine intended for jet injection.* (i) A conspicuous statement that the vaccine is intended for administration by jet injector.

(ii) A statement that the vaccine has been shown by appropriate test methods to contain not more than one organism per 100 doses or reference to an enclosed circular that contains such